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APPLICATION FOR UNITED STATES LETTERS PATENT

for

SHORT BIOACTIVE PEPTIDES

by

Donald R. Owen

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FIELD OF THE INVENTION

The invention relates to short length peptides containing phenylalanine, leucine, alanine, and lysine amino acid residues (F, L, A, and K; "FLAK peptides") in their primary sequence. In particular, FLAK peptides having desirable antimicrobial, antifungal, anticancer, and other biological activities are disclosed.

BACKGROUND OF THE INVENTION

Various bioactive peptides have been reported in both the scientific literature and in issued patents. Peptides historically have been isolated from natural sources, and have recently been the subject of structure-function relationship studies. Additionally, natural peptides have served as starting points for the design of synthetic peptide analogs.

A review of peptide antibiotics was published by R.E.W. Hancock in 1997 (*Lancet* 349: 418-422). The structure, function, and clinical applications of various classes of peptides were discussed. An additional review of cationic peptide antibiotics was published in 1998 (Hancock, R.E.W. and Lehrer, R. *Trends Biotechnol.* 16: 82-88). The peptides are typically cationic amphipathic molecules of 12 to 45 amino acids in length. The peptides permeabilize cell membranes leading to the control of microbial agents. The clinical potential of host defense cationic peptides was discussed by R.E.W. Hancock in 1999 (*Drugs* 57(4): 469-473; *Antimicrobial Agents and Chemotherapy* 43(6): 1317-1323). The antibacterial, antifungal, antiviral, anticancer, and wound healing properties of the class of peptides are discussed.

Reviews of the structural features of helical antimicrobial peptides, and their presumed mechanisms of action have been published (see, for example, Dathe, M. and Wieprecht, T. *Biochimica et Biophysica Acta* 1462: 71-87 (1999); Epand, R.M. and Vogel H.J. *Biochimica et Biophysica Acta* 1462: 11-28 (1999)). Structural parameters believed to be capable of modulating activity and selectivity include helicity, hydrophobic moment, hydrophobicity, angle subtended by the hydrophilic/hydrophobic helix surfaces, and charge.

A wide array of naturally occurring alpha helical peptides have been reported. The following are representative of the many references in the field.

Cecropins are a family of α-helical peptides isolated from insects. Cecropins are known for their antibacterial properties, as described in U.S. Patent Nos. 4,355,104 and 4,520,016. The cecropins were generally found to have activity against gram-negative bacteria, but not against all gram-negative bacteria. Cecropins were found not to have activity against eucaryotic cells (Andreu, et al., *Biochemistry* 24: 163-188 (1985); Boman, et al., *Developmental and Comparative Immunol.* 9: 551-558 (1985); Steiner et al., *Nature* 292: 246-248 (1981)). Cecropins from *Drosophila* and *Hyalphora* were presented as having activity against various strains of fungi (Ekengren, S. and Hultmark, D., *Insect Biochem. and Molec. Biol.* 29: 965-972 (1999)). Cecropin A from mosquito *Aedes aegypti* is reportedly different from most insect cecropins in that it lacks tryptophan and C-terminal amidation (Lowenberger, C. et al., *J. Biol. Chem.* 274(29): 20092-20097 (1999)).

Frogs from the genus *Rana* produce a wide array of antimicrobial peptides in their skin (Goraya, J. et al., *Eur. J. Biochem.* 267: 894-900 (2000)). Peptides as short as 13 amino acids were reported, and were grouped into structural families. The sequences showed little or no sequence identity to peptides isolated from frogs of other genera, such as the magainin and dermaseptin peptides.

U.S. Patent No. 5,962,410 disclosed the inhibition of eucaryotic pathogens, and the stimulation of lymphocytes and fibroblasts with lytic peptides such as cecropins and sarcotoxins. Various peptides presented include Cecropin B, Cecropin SB-37, Cecropin A, Cecropin D, Shiva-1, Lepidopteran, Sarcotoxin 1A, Sarcotoxin 1B, and Sarcotoxin 1C.

Transgenic mice producing the Shiva-1 cecropin class lytic peptide were reported by Reed, W.A. et al., *Transgenic Res.* 6: 337-347 (1997). Infection of the transgenic mice with a *Brucella abortus* challenge resulted in a reduction of the number of bacteria relative to infection of non-transgenic mice.

Magainin is an α-helical 23 amino acid peptide isolated from the skin of the African frog *Xenopus laevis* (Zasloff, M. *Proc. Natl. Acad. Sci. U.S.A.* 84: 5449-5453 (1987).

Cathelin associated α-helical peptides of 23 to 38 amino acids are found in the blood cells of sheep, humans, cattle, pigs, mice, and rabbits (Zanetti, M. et al., *FEBS Lett.* 374: 1-5 (1995)).

The antimicrobial activities of buforin II, cecropin P1, indolicidin, magainin II, nisin, and ranalexin were reported by Giacomette, A. et al. (*Peptides* 20: 1265-1273 (1999)). The peptides showed variable activities against bacteria and yeast.

Various synthetic peptides have been prepared and assayed both *in vitro* and *in vivo*.

U.S. Patent No. 5,861,478 disclosed synthetic lytic peptides of about 20 to 40 amino acids which adopt an α-helical conformation. The peptides are effective in the treatment of microbial infections, wounds, and cancer. The peptides disclosed include cecropin B, SB-37*, LSB-37, SB-37, Shiva 1 and 10-12, β-fibrin signal peptide, Manitou 1-2, Hecate 1-3, Anubis 1-5 and 8, and Vishnu 1-3 and 8.

Hecate was described as a synthetic peptide analog of melittin by Baghian, A. et al. (*Peptides* 18(2): 177-183 (1997)). The peptides differ in their charge distribution, but not in their amphipathic alpha helical conformation. Hecate inhibited herpes simplex virus (HSV-1) while not adversely affecting cell growth and protein synthesis.

Synthetic peptides D2A21, D4E1, D2A22, D5C, D5C1, D4E, and D4B were described in Schwab, U. et al., *Antimicrob. Agents and Chemotherapy* 43(6): 1435-1440 (1999). Activities against various bacterial strains were presented.

Hybrid peptides made of cecropin and melittin peptides were reportedly prepared and assayed by Juvvadi, P. et al. (*J. Peptide Res.* 53: 244-251 (1999)). Hybrids were synthesized to investigate the effects of sequence, amide bond direction (helix dipole), charge, amphipathicity, and hydrophobicity on channel forming ability and on antibacterial activity. Sequence and amide bond direction were suggested to be important structural requirements for the activity of the hybrids.

A 26 amino acid insect cecropin - bee melittin hybrid, and analogs thereof, were described in a study of salt resistance (Friedrich, C. et al., *Antimicrobial Agents and Chemotherapy* 43(7): 1542-1548 (1999)). A tryptophan residue in the second position was found to be critical for activity. Modest changes in sequence were found to lead to substantial changes in the properties of the peptides.

The effects of proline residues on the antibacterial properties of α-helical peptides has been published (Zhang, L. et al., *Biochem.* 38: 8102-8111 (1999)). The addition of prolines was reported to change the membrane insertion properties, and the replacement of a single proline may change an antimicrobial peptide into a toxin.

A series of peptides having between 18 and 30 amino acids were prepared in order to test the effects of changes in sequence and charge on antibacterial properties (Scott, M.G., et al., *Infect. Immun.* 67(4): 2005-2009 (1999)). No significant correlation was found between length, charge, or hydrophobicity and the antimicrobial activity of the peptides. A general trend was found that shorter peptides were less active than longer peptides, although the authors expressed that this effect would probably be sequence dependent.

"Modellins", a group of synthetic peptides were prepared and assayed to compare sequence and structure relationships (Bessalle, R. et al. *J. Med. Chem.* 36: 1203-1209 (1993)). Peptides of 16 and 17 amino acids having hydrophobic and hydrophilic opposite faces were highly hemolytic and antibacterial. Smaller peptides tended to have lower biological activities.

A cecropin-melittin hybrid peptide and an amidated flounder peptide were found to protect salmon from *Vibrio anguillarum* infections *in vivo* (Jia, X. et al., *Appl. Environ. Microbiol.* 66(5): 1928-1932 (2000)). Osmotic pumps were used to deliver a continuous dose of either peptide to the fish.

Amphipathic peptides have been reported as being capable of enhancing wound healing and stimulating fibroblast and keratinocyte growth *in vivo* (U.S. Patent Nos. 6,001,805 and 5,561,107). Transgenic plants have been reportedly prepared expressing lytic peptides as a fusion protein with ubiquitin (U.S. Patent No. 6,084,156). Methylated lysine rich lytic peptides were reportedly prepared, displaying improved proteolytic resistance (U.S. Patent No. 5,717,064).

While a number of natural and synthetic peptides exist, there exists a need for improved bioactive peptides and methods for their use.

SUMMARY OF THE INVENTION

Short (i.e. no more than 23 amino acids in length) peptides containing phenylalanine, leucine, alanine, and lysine amino acid residues in their primary sequence are disclosed. The peptides display desirable antibacterial, antifungal, anticancer biological activities, and also cause stimulation and proliferation of human fibroblasts and lymphocytes.

DESCRIPTION OF THE SEQUENCE LISTINGS

The following sequence listings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these sequences in combination with the detailed description of specific embodiments presented herein.

Table 1

SEQ ID NO:	Name	P-No.	Primary sequence
1	Hecate AC #1010	1	FALALKALKKALKKLKKALKKAL-COOH
2	Hecate AM	2	FALALKALKKALKKLKKALKKAL-NH2
3	SB-37 AC #1018	5	MPKWKVFKKIEKVGRNIRNGIVKAGPAIAVLGEAKALG-COOH
4	Shiva 10 AM	11	FAKKLAKKLKKLAKKLAKLALAL-NH2
5	SB-37 AM	12	MPKWKVFKKIEKVGRNIRNGIVKAGPAIAVLGEAKALG-NH2
6	Shiva 10 AC #1015	13	FAKKLAKKLKKLAKKLAKLALAL-COOH
7	Magainin 2	16	GIGKFLHSAKKFGKAFVGGIMNS-NH2
8	FLAK01 AM	23	FALAAKALKKLAKKLKKLAKKAL-NH2
9	FLAK03 AM	24	FALALKALKKLLKKLKKLAKKAL-NH2
10	FLAK04 AM	25	FALALKALKKLAKKLKKLAKKAL-NH2
11	FLAK05 AM	26	FALAKLAKKAKAKLKKALKAL-NH2
12	FLAK06 AM	27	FALALKALKKLKKALKKAL-NH2
13	FLAK06 AC	27 B	FALALKALKKLKKALKKAL-COOH
14	FLAK06 R-AC	27 C	FAKKLAKKLKKLAKLALAL-COOH
15	KAL V	30	VALALKALKKALKKLKKALKKAL-NH2
16	FLAK 17 AM	34	FALALKKALKALKKAL-NH2
17	FLAK 26 AM	35	FAKKLAKLAKKLAKLAL-NH2
18	FLAK 25 AM	36	FAKKLAKLAKKLAKLALAL-NH2
19	Hecate 2DAc	37	FALALKALKKAL-(D)-K-(D)-KLKKALKKAL-COOH
20	FLAK43 AM	38	FAKKLAKLAKKLLAL-NH2
21	FLAK44 AM	39	FAKKLAKLAKKALAL-NH2
22	FLAK62 AM	40	FALAKKALKKAKKAL-NH2

23	FLAK 06R-AM	41	FAKKLAKKLKKLAKLALAK-NH2
24	MSI-78 AM	42	GIGKFLKKAKKFGKAFVKILKK-NH2
25	FLAK50	43	FAKLLAKLAKKLL-NH2
26	FLAK51	44	FAKKLAKLALKLAKL-NH2
27	FLAK57	45	FAKKLAKKLAKLAL-NH2
28	FLAK71	46	FAKKLKKLAKLAKKL-NH2
29	FLAK77	47	FAKKALKALKKL-NH2
30	FLAK50V	48	VAKLLAKLAKKLL-NH2
31	FLAK50F	49	FAKLLAKLAKKL-NH2
32	FLAK26V AM	50	VAKKLAKLAKKLAKLAL-NH2
33	CAME-15	53	KWKLFKKIGAVLKVL-NH2
34	FLAK50C	54	FAKLLAKLAKKAL-NH2
35	FLAK50D	55	FAKLLAKALKKLL-NH2
36	FLAK 50E	56	FAKLLKLAAKKLL-NH2
37	FLAK80	57	FAKLLAKKLL-NH2
38	FLAK81	58	FAKKLAKALL-NH2
39	FLAK82	59	FAKKLAKKLL-NH2
40	FLAK83M	60	FAKLAKKLL-NH2
41	FLAK 26 Ac	61	FAKKLAKLAKKLAKLAL-COOH
42	Indolicidin	63	ILPWKWPWWPWRR-NH2
43	FLAK 17C	64	FAKALKALLKALKAL-NH2
44	FLAK 50H	65	FAKLLAKLAKAKL-NH2
45	FLAK 50G	66	FAKLLAKLAKLKL-NH2
46	Shiva Deriv P69+KWKL	70	FAKKLAKKLKKLAKKLAKKWKL-NH2
47	Shiva 10 (1-18 AC)	71	FAKKLAKKLKKLAKKLAK-COOH
48	Shiva 10 peptide 71+KWKL	72	FAKKLAKKLKKLAKKLAKKWKL-COOH
49	CA(1-7)Shiva10(1-16)	73	KWKLFKKKTKLFKKFAKKLAKKL-NH2
50	FLAK 54	74	FAKKLAKKLAKAL-NH2
51	FLAK 56	75	FAKKLAKKLAKLL-NH2
52	FLAK 58	76	FAKKLAKKLAKAAL-NH2
53	FLAK 72	77	FAKKLAKKAKLAKKL-NH2
54	FLAK 75	79	FAKKLKKLAKKL-NH2
55	Shiva 10 (1-16) Ac	80	KTKLFKKFAKKLAKKLKKLAKKL-COOH
56	CA(1-7)Shiva10 (1-16)-COOH	81	KWKLFKKKTKLFKKFAKKLAKKL-COOH
57	Indolocidin-ac	91	ILPWKWPWWPWRR-COOH
58	FLAK50B	92	FAKALAKLAKKLL-NH2
59	FLAK50J	93	FAKLLAKLAKKAA-NH2
60	FLAK50I	94	FAKLLALALKLKL-NH2
61	FLAK50K	95	FAKLLAKLAKAKA-NH2
62	FLAK50L	96	FAKLLAKLAKAKG-NH2
63	Shiva-11	98	FAKKLAKKLKKLAKKLAKLALALKALALKAL-NH2
64	Shiva 11 [(1-16)ME(2-9] - COOH	99	FAKKLAKKLKKLAKKLIGAVLKV-COOH
65	FLAK 50N	101	FAKLLAKALKLKL-NH2
66	FLAK 50O	102	FAKLLAKALKKAL-NH2
67	FLAK 50P	103	FAKLLAKALKKL-NH2
68	CA(1-&Hecate(11/23)	104	KWKLFKKALKKLKKALKKAL-NH2

69	PYL-ME	105	KIAKVALAKLGIGAVLKVLTTGL-NH2
70	FLAG26-D1	106	FAKKLAKLAKKL-NH2
71	Vishnu3	107	MPKEKVFLKIEKMGRNIRN-NH2
72	Melittin	108	GIGAVLKVLTTGLPALISWIKRKRQQ-NH2
73	FLAK26-D2	109	FAKKLAKLAKKLAKAL-NH2
74	FLAG26-D3	110	FAKKLLAKALKL-NH2
75	FLAK50 Q1	111	FAKFLAKFLKKAL-NH2
76	FLAK50 Q2	112	FAKLLFKALKKAL-NH2
77	FLAK50 Q3	113	FAKLLAKFLKKAL-NH2
78	FLAK50 Q4	114	FAKLLAKAFKKAL-NH2
79	FLAK50 Q5	117	FAKLFAKAFKKAL-NH2
80	FLAK50 Q6	118	FAKLLAKALKKFL-NH2
81	FLAK50 Q7	119	FAKLLAKALKKFAL-NH2
82	FLAK50 Q8	120	FAKLLAKLAKKFAL-NH2
83	FLAK50 Q9	121	FAKLFAKLAKKFAL-NH2
84	FLAK50 Q10	122	FKLAFKLAKKAFL-NH2
85	FLAK50 T1	123	FAKLLAKLAK-NH2
86	FLAK50 T2	124	FAKLLAKLAKKVL-NH2
87	FLAK50 T3	125	FAKLLAKLAKKIL-NH2
88	FLAK50 T4	126	FAKLLAKLAKKEL-NH2
89	FLAK50 T5	127	FAKLLAKLAKKSL-NH2
90	FLAK90	128	FAKLA-NH2
91	FLAK91	129	FAKLF-NH2
92	FLAK92	130	KAKLF-NH2
93	FLAK93	131	KWKLF-NH2
94	FLAK50 Z1	132	FGKGIGKVGKKLL-NH2
95	FLAK50 Z2	133	FAFGKGIGKVGKKLL-NH2
96	FLAK50 Z3	134	FAKAIAKIAFGKGIGKVGKKLL-NH2
97	FLAK50 Z4	135	FAKLWAKLAFGKGIGKVGKKLL-NH2
98	FLAK50 Z5	136	FAKLWAKLAKKL-NH2
99	FLAK50 Z6	137	FAKGVGKVGKKAL-NH2
100	FLAK50 Z7	138	FAFGKGIGKIGKKGL-NH2
101	FLAK50 Z8	139	FAKIIAKIAKIAKKIL-NH2
102	FLAK50 Z9	140	FAFAKIIAKIAKKII-NH2
103	FLAK94	141	FALALKA-NH2
104	FLAK93B	142	KWKLAKKALALL-NH2
105	FLAK50 Z10	143	FAKIIAKIAKKI-NH2
106	FLAK96	144	FALALKALKKAL-NH2
107	FLAK97	145	FALKALKK-NH2
108	FLAK98	146	KYKKALKKLAKLL-NH2
109	FKRLA	147	FKRLAKIKVLRLAKIKR-NH2
110	FLAK91B	148	FAKLAKKALAKLL-NH2
111	FLAK92B	149	KAKLAKKALAKLL-NH2
112	FLAK99	150	KLALKLALKALKAAKLA-NH2
113	FLAK50T6	151	FAKLLAKLAKK-NH2
114	FLAK50T7	152	FAKLLAKLAKKGL-NH2
115	FLAK95	153	FALKALKKLKKALKKAL-NH2
116	FLAK50T8	154	VAKLLAKLAKKVL-NH2
117	FLAK50T9	155	YAKLLAKLAKKAL-NH2
118	FLAK100-CO2H	156	KLLKLLLKLYKKLLKLL-COOH
119	FAGVL	157	FAVGLRAIKRALKKLRRGVRKVAKDL-NH2
120	Modelin-5	159	KLAKKLAKLAKLAKAL-NH2

121	Modelin-5-CO2H	160	KLAKKLAKLAKLAKAL-COOH
122	Modelin-8	161	KWKKLAKKW-NH2
123	Modelin-8-CO2H	162	KWKKLAKKW-COOH
124	Modelin-1	163	KLWKKWAKKWLKLWKAW-NH2
125	Modelin-1-CO2H	164	KLWKKWAKKWLKLWKA-COOH
126	FLAK120	165	FALALKALKKL-NH2
127	FLAK121	166	FALAKALKKAL-NH2
128	FLAK96B	167	FALALKLAKKAL-NH2
129	FLAK96G	168	FALLKL-NH2
130	FLAK96F	169	FALALKALKK-NH2
131	FLAK96C	170	FALKALKKAL-NH2
132	FLAK96D	171	FALLKALKKAL-NH2
133	Modelin-8B	172	KWKK-NH2
134	Modelin-8C	173	KWKKL-NH2
135	Modelin-8D	174	KFKKLAKKF-NH2
136	Modelin-8E	175	KFKKLAKKW-NH2
137	Flak 96	176	FALALKALKKA-NH2
138	Flak 96I	177	FALLKALLKKAL-NH2
139	Flak 96J	178	FALALKLAKKL-NH2
140	Flak 96L	179	LKKLAKLALAF-NH2
141	FLAK-120G	180	VALALKALKKL-NH2
142	FLAK-120D	181	FALALKLKKL-NH2
143	FLAK-120C	182	FALALKAKKL-NH2
144	FLAK-120B	183	FALA-NH2
145	FLAK-120F	184	WALAL-NH2
146	Magainin2wisc	300	GIGKFLHAAKKFAKAFVAEIMNS-NH2
147	D2A21	301	FAKKFAKKFKKFAKKFAKFAFAF-NH2
148	KSL-1	302	KKVVFKVKFK-NH2
149	KSL-7	303	FKVKFKVKVK-NH2
150	LSB-37	306	LPKWKVFKKIEKVGRNIRNGIVKAGPAIAVLGEAKALG-NH2
151	Anubis-2	307	FAKKLAKKLKKLAKKLAKLAKKL-NH2
152	FLAK17CV	501	VAKALKALLKALKAL-NH2
153	FLAK50Q1V	502	VAKFLAKFLKKAL-NH2
154	D2A21V	503	VAKKFAKKFKKFAKKFAKFAFAF-NH2
155	FLAK25AMV	504	VAKKLAKLAKKLAKLALAL-NH2
156	FLAK43AMV	505	VAKKLAKLAKKLLAL-NH2
157	FLAK50DV	506	VAKLLAKALKKLL-NH2
158	HECATE AMV	507	VALALKALKKALKKLKKALKKAL-NH2
159	HECATE ACV	508	VALALKALKKALKKLKKALKKAL-COOH
160	FLAK04AMV	509	VALALKALKKLAKKLKKLAKKAL-NH2
161	FLAK03AMV	510	VALALKALKKLLKKLKKLAKKAL-NH2
162	D-Shiva 10 AC	67	(D)-FAKKLAKKLKKLAKKLAKLALAL-COOH
163	Shiva 11 AC	100	FAKKLAKKLKKLAKKLAKLALALKALALKA-COOH
164	Shiva 10 (1-18)AM	69	FAKKLAKKLKKLAKKLAK-NH2
165	FLAK 50M	97	FAKLLALALKKAL-NH2

DETAILED DESCRIPTION OF THE INVENTION

The invention is generally directed towards peptides having desirable biological properties, and their use. It is surprising that the peptides are efficacious due to their short length as compared to other peptides described in the art.

Peptides

One embodiment of the invention is directed towards an isolated peptide comprising phenylalanine, leucine, alanine, and lysine residues, wherein the peptide is about 5 to about 23 amino acids in length. The peptide can have a minimum length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or about 18 amino acids. The peptide can have a maximum length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or about 23 amino acids. The peptide can be about 5 to about 20 amino acids in length. The peptide can consist essentially of, or consist of phenylalanine, leucine, alanine, and lysine residues. The peptide can have a percent amino acid composition of phenylalanine, leucine, alanine, and lysine residues of at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. The peptide can generally be any of the listed SEQ ID NOS which fall within these various guidelines, and more preferably is SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:112, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:137, SEQ ID NO:138, SEQ ID

NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:152, SEQ ID NO:159, SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO:164, and SEQ ID NO:165. The peptide is preferably not hecate-1, anubis-1, anubis-2, anubis-5, anubis-8, vishnu-1, vishnu-2, vishnu-3, vishnu-8, or shiva-10.

The peptide can be similar to any of the above described peptides, and preferably is similar to SEQ ID NO:2 (or SEQ ID NO:16 or SEQ ID NO:126), SEQ ID NO:4 (or SEQ ID NO:14 or SEQ ID NO:17), SEQ ID NO:25, SEQ ID NO:43, SEQ ID NO:75, SEQ ID NO:84, SEQ ID NO:115, or SEQ ID NO:132 as determined by percent identity. The percent identity between the peptides is preferably at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. Percent identity is determined using a sequence alignment by the commercial product CLUSTALW. The number of aligned amino acids are divided by the length of the shorter peptide, and the result is multiplied by 100% to determine percent identity. If the length of the shorter peptide is less than 10 amino acids, the number of aligned amino acids are divided by 10, and the result is multiplied by 100% to determine percent identity.

The peptides can comprise D- or L- amino acids. The peptides can comprise all D- amino acids. The peptides can have an acid C-terminus (-CO_2H) or an amide C-terminus (-$CONH_2$, -CONHR, or -$CONR_2$).

Methods of use

An additional embodiment of the invention is directed towards methods of using the above described peptides. The methods of use preferably do not cause injury or kill normal uninfected mammalian cells. The methods of use at therapeutic dose levels preferably do not cause injury to or kill normal uninfected or non-neoplastic mammalian cells. The methods of use may involve the use of a single peptide, or may involve the use of multiple peptides.

An embodiment of the invention is the use of the above described peptides to inhibit or kill microbial cells (microorganisms). The microorganisms may be bacterial cells, fungal cells, protozoa, viruses, or eucaryotic cells infected with pathogenic microorganisms. The method generally is directed towards the contacting of microorganisms with the peptide. The contacting step can be performed *in vivo*, *in vitro*,

topically, orally, transdermally, systemically, or by any other method known to those of skill in the art. The contacting step is preferably performed at a concentration sufficient to inhibit or kill the microorganisms. The concentration of the peptide can be at least about 0.1 μM, at least about 0.5 μM, at least about 1 μM, at least about 10 μM, at least about 20 μM, at least about 50 μM, or at least about 100 μM. The methods of use can be directed towards the inhibition or killing of microorganisms such as bacteria, gram positive bacteria, gram negative bacteria, mycobacteria, yeast, fungus, algae, protozoa, viruses, and intracellular organisms. Specific examples include, but are not limited to, *Staphylococcus*, *Staphylococcus aureus*, *Pseudomonas*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Chlamydia*, *Candida albicans*, *Saccharomyces*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Trypanosoma cruzi*, or *Plasmodium falciparum*. The contacting step can be performed by systemic injection, oral, subcutaneous, IP, IM, IV injection, or by topical application. For injection, the dosage can be between any of the following concentrations: about 1 mg/kg , about 5 mg/kg, about 10 mg/kg, about 25 mg/kg, about 50 mg/kg, about 75 mg/kg, and about 100 mg/kg. The contacting step can be performed on a mammal, a cat, a dog, a cow, a horse, a pig, a bird, a chicken, a plant, a fish, or a human.

Preferred peptides for antibacterial applications include SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:93, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:112, SEQ ID NO:115, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO:164, and SEQ ID NO:165.

Preferred peptides for antifungal applications include SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:25, SEQ ID NO:30, SEQ ID NO:35, SEQ ID NO:58, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:131, SEQ ID NO:143, SEQ ID NO:163, and SEQ ID NO:165.

An additional embodiment of the invention is the use of any of the above described peptides to inhibit or kill cancer cells. The method generally is directed towards the contacting of cancer cells with the peptide. The contacting step can be performed *in vivo*, *in vitro*, topically, orally, transdermally, systemically, or by any other method known to those of skill in the art. The contacting step is preferably performed at a concentration sufficient to inhibit or kill the cancer cells. The concentration of the peptide can be at least about at least about 0.1 μM, at least about 0.5 μM, at least about 1 μM, at least about 10 μM, at least about 20 μM, at least about 50 μM, or at least about 100 μM. The cancer cells can generally be any type of cancer cells. The cancer cells can be sarcomas, lymphomas, carcinomas, leukemias, breast cancer cells, colon cancer cells, skin cancer cells, ovarian cancer cells, cervical cancer cells, testicular cancer cells, lung cancer cells, prostate cancer cells, and skin cancer cells. The contacting step can be performed by subcutaneous, IP injection, IM injection, IV injection, direct tumor injection, or topical application. For injection, the dosage can be between any of the following concentrations: about 0.1 mg/kg, about 1 mg/kg , about 5 mg/kg, about 10 mg/kg, about 25 mg/kg, about 50 mg/kg, about 75 mg/kg, and about 100 mg/kg. The contacting step can be performed on a mammal, a cat, a dog, a cow, a horse, a pig, a bird, a chicken, a plant, a fish, a goat, a sheep, or a human. The inhibition of cancer cells can generally be any inhibition of growth of the cancer cells as compared to the cancer cells without peptide treatment. The inhibition is preferably at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, and ideally 100% inhibition of growth. The inhibition may be achieved by lysis of the cancer cells or by other means. The cancer inhibiting peptide can be used synergistically with other cancer chemotherapeutic agents.

Preferred peptides for anticancer applications include SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:46, SEQ ID NO:51, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:68, SEQ ID NO:75, SEQ ID NO:86, SEQ ID NO:152, and SEQ ID NO:162

An additional embodiment of the invention is directed towards a method for promoting the stimulation and/or proliferation of cells. The method can comprise contacting the cells and a composition, wherein the composition comprises a peptide. The peptide can be any of the above described peptides. The concentration of the peptide in the composition can be about 0.01 μM to about 500 μM, about 0.1 μM to about 100 μM, about 1 μM to about 50 μM, or about 1 μM to about 10 μM. The cells can generally be any type of cells, and preferably are mammalian cells, specifically including, but not limited to fibroblast and leukocyte cells, including lymphocyte and phagocytic cells. The metabolic stimulation and/or proliferation of the cells is preferably increased by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 125%, 150%, 175%, or 200% relative to the same cells not contacted with the composition. The composition can further comprise a growth factor. The stimulatory and proliferative properties of some of the FLAK peptides hold promise for their application in skin care, wound healing, and in immunomodulation of compromised mammalian immune systems.

Preferred peptides for stimulation and proliferation applications include SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:87, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:132, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID

NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:159, SEQ ID NO:162, and SEQ ID NO:164.

An additional embodiment of the invention is directed towards a method for promoting wound healing of skin or ocular and internal body tissues damaged by normal aging, disease, injury, or by surgery or other medical procedures. The method can comprise administering to the wound of an animal a composition, wherein the composition comprises any of the above described peptides. The concentration of the peptide in the composition can be about 0.01 μM to about 500 μM, about 0.1 μM to about 100 μM, about 1 μM to about 50 μM, or about 1 μM to about 10 μM. The composition can be administered to the wound topically or by systemic delivery. The animal can generally be any kind of animal, preferably is a mammal, and more preferably is a human, cow, horse, cat, dog, pig, goat, or sheep. The promotion of wound healing is preferably at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 125%, 150%, 175%, or 200% relative to the same wound not contacted with the composition.

Preferred peptides for wound healing applications include SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:87, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:132, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:159, SEQ ID NO:162, and SEQ ID NO:164.

A further embodiment of the invention is directed towards methods for the additive or synergistic enhancement of the activity of a therapeutic agent. The method can comprise preparing a composition, wherein the composition comprises a peptide and a therapeutic agent. Alternatively, the method may comprise co-therapy treatment with a

peptide (or peptides) used in conjunction with other therapeutic agents. The peptide can be any of the above described peptides. The therapeutic agent can generally be any therapeutic agent, and preferably is an antibiotic, an antimicrobial agent, a growth factor, a chemotherapy agent, an antimicrobial agent, lysozyme, a chelating agent, or EDTA. Preferably, the activity of the composition is higher than the activity of the same composition containing the therapeutic agent but lacking the peptide. The composition or co-therapy can be used in *in vitro*, *in vivo*, topical, oral, IV, IM, IP, and transdermal applications. The enhancement of the activity of the composition containing the therapeutic agent and the peptide is preferably at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 125%, 150%, 175%, or 200% relative to the activity of the therapeutic agent alone.

Generally, any peptide which is active on a stand-alone basis against a target is preferred for use to increase either additively or synergistically the activity of another therapeutic agent against that target. If several peptides are candidates for a given synergy application, then the less toxic peptides would be more favorably considered.

The following Examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLES

Example 1: Antimicrobial assays

The data for the antimicrobial assay of the peptides have been obtained by making OD measurements in *in vitro* cell culture experiments with and without added peptide. The protocol used is as follows.

Cell lines included *Staphylococcus aureus* ATCC 6538 or 25923, *Pseudomonas aeruginosa* ATCC 9027 or 29853. Medium used were Antibiotic Medium 3 (Difco), Antibiotic Medium 2 (Difco), and 0.85% saline. Controls used were physiological saline, and gentamycin at 50, 25, 10, 5, 1, and 0.1 ppm.

The preparation of all media, stock solutions, and dilutions took place in a laminar flow hood to prevent contamination. Bacterial cells were freshly grown on antibiotic medium 2 agar slants (pH 7.0 at 25 °C). Bacteria were suspended and diluted in antibiotic medium 3 to about 10^4 cfu/ml and used as the inoculum. Sample solutions (100 µl/well) were added to plates according to the plate layout. Inoculum (100 µl/well) was added to achieve a final concentration of 5×10^3 cfu/ml. Negative controls received 100 µl saline and 100 µl growth medium. Positive controls received 100 µl saline and 100 µl inoculum. Bacterial plates were incubated at 37 °C for 24 hours.

Absorbance was read at 620 nm after shaking to resuspend cells. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of peptide that completely inhibits the growth of the test organism.

The yeast assay was performed in RPMI 1640 media (pH 7.0 at 25 °C).

The data presented in Table 2 were obtained using the above protocol. However, the data for Table 3 were obtained with a modified protocol wherein the medium was tryptic soy broth, inocolum strength was approximately 10^4 CFU per ml, and values determined were minimum bactericidal concentrations (MBC) or minimum fungicidal concentrations (MFC).

The following Table 2 describes the antimicrobial properties of the peptides measured as MIC or MFC values in µg/mL. Staph6538 is *Staphylococcus aureus* ATCC accession number 6538; paerug9027 is *Pseudomonas aeruginosa* ATCC accession number 9027, yeast is *Saccharomyces cerevisiae*.

Table 2

Name	SEQ ID NO:	P Number	staph6538	paerug9027	yeast
Hecate AC #1010	1	1	5	10	>
Hecate AM	2	2	25	100	25
SB-37 AC #1018	3	5	100	50	>
SB-37 AM	5	12	>	100	>

Shiva 10 AC #1015	6	13	10	>	>
FLAK01 AM	8	23	5	50	100
FLAK04 AM	10	25	10	5	25
FLAK05 AM	11	26	10	15	>
FLAK06 AM	12	27	10	10	25
KAL V	15	30	>	>	ND
FLAK 17 AM	16	34	5	50	25
FLAK 26 AM	17	35	5	200	25
Hecate 2DAc	19	37	5	100	50
FLAK43 AM	20	38	5	50	50
FLAK44 AM	21	39	100	25	100
FLAK62 AM	22	40	100	25	100
FLAK 06R-AM	23	41	10	10	ND
MSI-78 AM	24	42	10	>	200
FLAK50	25	43	5	100	25
FLAK51	26	44	5	5	50
FLAK57	27	45	5	100	100
FLAK71	28	46	10	5	50
FLAK77	29	47	200	100	50
FLAK50V	30	48	5	5	25
FLAK50F	31	49	10	200	50
FLAK26V AM	32	50	5	15	50
CAME-15	33	53	5	15	50
FLAK50C	34	54	5	50	50
FLAK50D	35	55	5	5	25
FLAK 50E	36	56	200	5	50
FLAK80	37	57	100	200	200
FLAK81	38	58	100	100	200
FLAK82	39	59	>	>	>
FLAK83M	40	60	200	100	200
FLAK 17 C	43	64	5	>	200
FLAK 50H	44	65	15	50	200
FLAK 50G	45	66	5	50	100
Shiva deriv P69+KWKL	46	70	10	>	100
Shiva 10 (1-18_ AC	47	71	15	15	200
CA(1-7)Shiva10(1-16)	49	73	50	15	100
FLAK 54	50	74	15	5	100
FLAK 56	51	75	5	5	50
FLAK 58	52	76	10	100	200
FLAK 72	53	77	200	100	200

FLAK 75	54	79	100	200	100
Shiva 10 (1-16) Ac	55	80	10	100	100
CA(1-7)Shiva10(1-16)-COOH	56	81	10	>	>
Indolocidin-ac	57	91	10	>	>
FLAK50B	58	92	5	5	50
FLAK50I	60	94	10	>	>
FLAK50K	61	95	100	200	>
FLAK50L	62	96	>	>	>
Shiva-11	63	98	>	>	>
Shiva 11[(1-16)ME(2-9)]-COOH	64	99	100	>	>
FLAK 50N	65	101	10	25	100
FLAK 50O	66	102	5	10	50
FLAK 50P	67	103	10	25	100
CA(1-&Hecate(11/23)	68	104	10	10	200
PYL-ME	69	105	200	200	>
FLAG26-D1	70	106	100	25	100
Vishnu3	71	107	>	>	>
Melittin	72	108	5	>	25
FLAK26-D2	73	109	>	200	200
FLAG26-D3	74	110	>	200	200
FLAK50 Q1	75	111	5	100	200
FLAK50 Q2	76	112	50	200	100
FLAK50 Q3	77	113	10	200	200
FLAK50 Q4	78	114	50	15	100
FLAK50 Q5	79	117	100	200	200
FLAK50 Q6	80	118	10	100	100
FLAK50 Q7	81	119	50	25	50
FLAK50 Q8	82	120	50	200	200
FLAK50 Q9	83	121	50	>	100
FLAK50 T1	85	123	50	200	100
FLAK50 T2	86	124	5	100	100
FLAK50 T3	87	125	10	100	50
FLAK50 T4	88	126	>	>	>
FLAK50 T5	89	127	100	25	100
FLAK90	90	128	>	100	200
FLAK91	91	129	100	25	100
FLAK92	92	130	200	200	200
FLAK93	93	131	25	10	100
FLAK50 Z1	94	132	>	100	>
FLAK50 Z2	95	133	>	>	>

FLAK50 Z3	96	134	100	>	200
FLAK50 Z4	97	135	15	10	50
FLAK50 Z5	98	136	100	50	100
FLAK50 Z6	99	137	>	>	>
FLAK50 Z7	100	138	>	>	>
FLAK50 Z8	101	139	50	25	200
FLAK50 Z9	102	140	>	>	>
FLAK94	103	141	15	50	200
FLAK93B	104	142	100	50	100
FLAK50 Z10	105	143	100	50	200
FLAK96	106	144	5	50	50
FLAK97	107	145	200	100	200
FLAK98	108	146	10	10	50
FKRLA	109	147	5	5	200
FLAK91B	110	148	>	200	200
FLAK92B	111	149	50	100	200
FLAK99	112	150	100	10	>
FLAK50T6	113	151	>	>	200
FLAK50T7	114	152	100	50	100
FLAK95	115	153	5	25	100
FLAK50T8	116	154	100	100	50
FLAK50T9	117	155	>	>	>
FLAK100-CO2H	118	156	15	>	>
FAGVL	119	157	200	>	>
FLAK120	126	165	10	25	25
FLAK121	127	166	>	>	>
FLAK96B	128	167	10	25	100
FLAK96G	129	168	50	100	>
FLAK96F	130	169	100	100	100
FLAK96C	131	170	200	100	100
FLAK96D	132	171	25	50	100
FLAK 96	137	176	>	>	>
FLAK 96J	139	178	200	100	>
FLAK 96L	140	179	50	50	100
FLAK-120G	141	180	200	>	>
FLAK-120D	142	181	100	200	100
FLAK-120C	143	182	>	>	>
FLAK-120B	144	183	200	100	200
FLAK-120F	145	184	25	100	100
FLAK 50M	165	97	5	50	50

> indicates greater than 200 μg/mL; ND = not determined.

The following Table 3 describes describes the antimicrobial properties of the peptides measured as minimum bactericidal or minimum fungicidal (*Candida*)

concentrations. MBC or MFC values are in µg/mL. E. coli is *Escherichia coli* ATCC accession number 25922; P. aerug is *Pseudomonas aeruginosa* ATCC accession number 27853, S. aur. is *Stapholococcus aureus* ATCC accession number 25923; Candida is *Candida albicans* ATCC accession number 10231.

Table 3

SEQ ID NO:	P #	E. coli A.25922	P.aerug A.27853	S.aur A.25923	Candida A.10231
1	1	25	30	25	>50
2	2	25	10	25	>50
3	5	50	>60	40	ND
4	11	40	25	25	>50
5	12	50	>60	75	ND
6	13	8	15	30	>50
8	23	15	25	30	>50
9	24	>80	30	>40	>50
10	25	40	30	40	>50
11	26	>80	>40	>40	>50
12	27	10	8	8	>50
13	27B	40	10	>40	>40
14	27C	10	4	>40	>40
15	30	10	15	40	>50
16	34	15	15	40	>40
17	35	8	8	10	>40
18	36	30	15	10	>40
19	37	8	8	40	>50
20	38	15	30	15	ND
21	39	>40	>40	>40	ND
22	40	30	40	>40	ND
23	41	40	40	40	ND
24	42	10	30	10	ND
25	43	8	15	4	15
26	44	10	55	30	>50
27	45	30	40	80	>50
29	47	>50	>50	>50	>50
30	48	8	25	4	10
31	49	40	30	50	30
32	50	50	25	25	>50
33	53	15	15	10	30
34	54	15	40	15	30
35	55	4	10	4	25
36	56	50	10	55	30
37	57	>50	>50	>50	>50

38	58	>50	>50	>50	>50
39	59	>50	>50	>50	>50
40	60	>50	>50	>50	>50
41	61	4	50	>80	>40
42	63	10	50	15	60
43	64	10	30	4	>50
44	65	>55	>50	>55	>50
45	66	40	50	30	40
46	70	40	30	40	>50
47	71	50	40	>50	>50
48	72	>50	40	>50	>50
50	74	>55	50	>55	>55
51	75	40	30	>55	30
52	76	40	>55	>55	>50
53	77	>50	>50	>50	>50
54	79	>50	>50	>50	>50
55	80	30	15	>50	>50
58	92	40	25	15	25
59	93	>50	>50	>50	>50
60	94	>50	>50	>50	>50
61	95	>50	>50	>50	>50
62	96	>50	>50	>50	>50
65	101	300	>50	>50	40
66	102	25	30	25	15
67	103	30	30	>50	25
69	105	25	>50	ND	>50
70	106	50	>50	ND	>50
71	107	ND	>50	>50	>50
72	108	>50	>50	25	>50
73	109	ND	ND	80	>50
74	110	8	>50	>50	>50
75	111	30	ND	40	INACT
76	112	30	INACT	INACT	INACT
77	113	INACT	INACT	INACT	40
79	117	INACT	INACT	INACT	INACT
80	118	8	25	10	25
81	119	15	30	4	25
82	120	INACT	INACT	INACT	INACT
83	121	INACT	INACT	INACT	50
84	122	30	30	25	15
85	123	40	INACT	INACT	25
86	124	10	40	8	15
87	125	40	40	INACT	40
88	126	INACT	INACT	INACT	INACT

89	127	INACT	INACT	INACT	INACT
90	128	INACT	INACT	INACT	INACT
91	129	INACT	INACT	INACT	INACT
92	130	INACT	INACT	INACT	INACT
93	131	INACT	INACT	INACT	INACT
94	132	INACT	INACT	INACT	INACT
95	133	INACT	INACT	INACT	INACT
96	134	INACT	INACT	INACT	25
97	135	INACT	40	INACT	INACT
98	136	INACT	INACT	INACT	INACT
99	137	INACT	INACT	INACT	INACT
100	138	INACT	INACT	INACT	INACT
101	139	INACT	INACT	INACT	INACT
102	140	INACT	INACT	INACT	INACT
103	141	INACT	INACT	INACT	INACT
104	142	INACT	INACT	INACT	INACT
105	143	INACT	INACT	INACT	INACT
106	144	10	25	25	25
107	145	INACT	INACT	INACT	100
108	146	10	>250	75	10
109	147	25	75	>250	>250
110	148	150	>250	>250	100
111	149	150	>250	>250	100
112	150	75	>250	>250	50
113	151	>250	>250	>250	100
114	152	150	150	>250	50
115	153	10	25	5	25
116	154	50	100	>250	25
117	155	>250	>250	>250	>250
118	156	100	>250	>250	>250
119	157	75	>250	>250	>250
120	159	10	10	>250	50
121	160	>250	>250	>250	>250
122	161	150	>250	>250	25
123	162	50	>250	>250	100
124	163	25	50	25	25
125	164	25	25	25	25
126	165	10	25	25	10
127	166	>250	>250	>250	>250
128	167	25	>250	10	25
129	168	75	100	>250	150
130	169	200	>250	>250	75
131	170	25	>250	150	25
132	171	75	100	>250	50

133	172	>250	>250	>250	>250
134	173	>250	>250	>250	150
162	67	25	30	30	>50
165	97	25	>50	25	25

INACT refers to no detectable activity. ND indicates no data available.

Example 2: Anti-cancer assays

Cancer cell assays were performed in a manner similar to the anti-microbial assays described above, except that the assay procedure used the MTT dye protocol. Viability of cells is determined by the dye response. In the following procedure, approximately 1.5 x 10^4 cells per well were added and viability was determined with the cells in a semi-confluent state. The assay was performed in a 96-well microtiter plate. After addition of peptide, the plate was set for 24 hours. MTT (5 mg/ml in phenol red-free RPMI-1640, 20 µl) was added to each well including positive control wells untreated with peptide. The plate was incubated at 37 °C for 4 hours. The liquid contents of each well was removed, and isopropanol with 0.1 M HCl (100 µl) was added to each well. The plate was sealed with parafilm to prevent evaporation of the isopropanol. The plate is allowed to rest for 5-10 minutes in order to solubilize the precipitate. Purified water (100 µl) was added to each well. Absorbance was determined with an ELISA Reader instrument. Color intensity at 540 nm is proportional to viability of cells. Results for each concentration of peptide are plotted relative to untreated controls, and LD50 values are determined from the graphs.

WI38 (ATCC No. CCL75) is a normal fibroblast line of lung diploid cells, MCF7 (ATCC No. HTB22) is a breast adenocarcinoma tumor cell line, SW480 (ATCC No. CCL228) is a colon adenocarcinoma tumor cell line, BMKC is a cloned melanoma line derived from Bowes melanoma line HMCB (ATCC No. CRL9607), H1299 (ATCC No. CRL5803) is a lung large cell carcinoma tumor line, HeLaS3 (ATCC No. CCL2.2) is a cervical epitheleal carcinoma tumor cell line, and PC3 (ATCC No. CRL1435) is a prostate adenocarcinoma tumor cell line. Numbers are LD_{50} values (µg/mL). Data on the six targets are presented in the following Tables 4 and 5.

Table 4

Name	SEQ ID NO:	P No.	WI38	MCF7	SW480	BMKC
HECATE AC	1	1	27	54	6	72
HECATE AM	2	2	66	23	46	128
SB37COOH	3	5	130	175	82	120
SB-37 AM	5	12	950	540	>	>
SHIVA 10 AC	6	13	57	>	ND	ND
FLAK01 AM	8	23	34	62	5	27
FLAK03 AM	9	24	55	26	38	85
FLAK04 AM	10	25	24	10	12	36
FLAK05 AM	11	26	96	74	8	94
FLAK06 AM	12	27	37	14	26	44
FLAK06 AC	13	27B	101	65	59	93
FLAK06 R-AC	14	27C	520	140	210	300
KAL V	15	30	93	72	62	140
FLAK 17 AM	16	34	40	21	35	53
FLAK 26 AM	17	35	8	9	14	7
FLAK 25 AM	18	36	19	9	30	56
HECATE 2DAc	19	37	80	14	57	150
FLAK43 AM	20	38	12	17	13	21
FLAK44 AM	21	39	300	130	435	510
FLAK62 AM	22	40	>	760	>	>
FLAK 06R-AM	23	41	175	98	120	290
MSI-78 AM	24	42	67	31	34	140
FLAK50	25	43	5	9	9	7
FLAK51	26	44	36	140	32	47
FLAK57	27	45	200	260	180	160
FLAK71	28	46	200	300	160	150
FLAK77	29	47	>	575	>	700
FLAK50V	30	48	41	23	47	43
FLAK50F	31	49	135	40	100	115
FLAK26V AM	32	50	43	32	46	40
CAME-15	33	53	32	45		40
FLAK50C	34	54	97	60		90
FLAK50D	35	55	32	16	14	16
FLAK 50E	36	56	250	500	215	205
FLAK80	37	57	900	>	740	740
FLAK81	38	58	>	>	>	>
FLAK82	39	59	77	31	42	155
FLAK83M	40	60	>	>	>	>
FLAK 26 Ac	41	61	93	105	100	140
INDOLICIDIN	42	63	ND	64	345	200

				80		35
FLAK 17 C	43	64	37	475	345	250
FLAK 50H	44	65	320	90	145	200
FLAK 50G	45	66	240	44	11	94
SHIVA DERIV P69+KWKL	46	70	34	190	250	445
SHIVA 10 (1-18_ AC	47	71	355	93	82	290
SHIVA 10 PEPTIDE 71+KWKL	48	72	125	150	70	360
CA(1-7)Shiva10(1-16)	49	73	160	465	340	460
FLAK 54	50	74	335	42	17	24
FLAK 56	51	75	80	970	400	750
FLAK 58	52	76	445	>	>	125
FLAK 72	53	77	>	540	>	830
FLAK 75	54	79	>	29	35	76
SHIVA 10 (1-16) Ac	55	80	28	63	13	12
CA(1-7)Shiva10(1-16)-COOH	56	81	8	12	30	180
INDOLOCIDIN-ac	57	91	9	23	51	46
FLAK50B	58	92	43	65	ND	11
FLAK50I	60	94	6	>	>	820
FLAK50K	61	95	250	>	>	>
FLAK50L	62	96	>	96	125	94
Shiva-11	63	98	47	95	120	94
SHIVA 11 [(1-16)ME(2-9] -COOH	64	99	34	250	170	160
FLAK 50N	65	101	300	60	57	60
FLAK 50O	66	102	73	46	90	75
FLAK 50P	67	103	26	11	54	100
CA(1-&HECATE(11/23)	68	104	24	635	>	ND
PYL-ME	69	105	430	620	570	690
FLAG26-D1	70	106	>	>	>	>
VISHNU3	71	107	>	9	23	18
MELITTIIN	72	108	16	>	>	>
FLAK26-D2	73	109	>	180	325	400
FLAG26-D3	74	110	45	35	27	26
FLAK50 Q1	75	111	24	500	800	445
FLAK50 Q2	76	112	420	150	180	115
FLAK50 Q3	77	113	170			

FLAK50 Q4	78	114	>	730	>	>
FLAK50 Q5	79	117	>	>	>	>
FLAK50 Q6	80	118	170	70	115	135
FLAK50 Q7	81	119	45	54	46	36
FLAK50 Q8	82	120	600	730	630	660
FLAK50 Q9	83	121	625	400	800	670
FLAK50 Q10	84	122	720	360	570	700
FLAK50 T1	85	123	600	615	>	635
FLAK50 T2	86	124	21	18	9	10
FLAK50 T3	87	125	90	90	125	220
FLAK50 T4	88	126	>	>	>	>
FLAK50 T5	89	127	760	440	400	535
FLAK90	90	128	500	500	530	330
FLAK91	91	129	>	>	550	>
FLAK92	92	130	>	>	>	>
FLAK93	93	131	>	600	555	>
FLAK50 Z1	94	132	>	>	>	>
FLAK50 Z2	95	133	>	>	>	>
FLAK50 Z3	96	134	>	>	740	>
FLAK50 Z4	97	135	110	54	80	155
FLAK50 Z5	98	136	>	500	600	530
FLAK50 Z6	99	137	>	>	>	>
FLAK50 Z7	100	138	>	>	>	>
FLAK50 Z8	101	139	550	625	>	525
FLAK50 Z9	102	140	>	>	>	>
FLAK94	103	141	420	430	560	465
FLAK93B	104	142	73	44	38	38
FLAK50 Z10	105	143	>	>	>	>
FLAK96	106	144	750	150	285	250
FLAK97	107	145	>	>	>	>
FLAK98	108	146	270	110	380	185
FKRLA	109	147	83	106	185	110
FLAK91B	110	148	380	315	>	330
FLAK92B	111	149	>	>	>	>
FLAK99	112	150	125	160	235	190
FLAK50T6	113	151	>	>	>	>
FLAK50T7	114	152	620	430	740	>
FLAK95	115	153	130	64	61	165
FLAK50T8	116	154	600	315	750	330
FLAK50T9	117	155	>	>	>	>
FLAK100-CO2H	118	156	230	135	345	520
FAGVL	119	157	500	240	530	600
Modelin-5	120	159	82	61	140	140
Modelin-5-CO2H	121	160	700	320	370	220

FLAK120	126	165	470	360	240	240
FLAK121	127	166	>	>	>	>
FLAK96B	128	167	260	230	360	240
FLAK96G	129	168	>	630	>	590
FLAK96F	130	169	>	510	>	530
FLAK96C	131	170	>	940	>	>
FLAK96D	132	171	615	305	770	600
Modelin-8D	135	174	>	>	>	>
Modelin-8E	136	175	>	>	70	>
Flak 96H	137	176	>	>	>	>
Flak 96I	138	177	270	190	310	310
Flak 96J	139	178	405	770	>	640
Flak 96L	140	179	540	555	>	920
FLAK-120G	141	180	940	950	600	770
FLAK-120D	142	181	500	550	870	830
FLAK-120C	143	182	>	>	>	>
FLAK-120B	144	183	>	>	>	>
FLAK-120F	145	184	800	260	440	600
Magainin2wisc	146	300	52	22	60	130
D2A21	147	301	66	64	76	140
KSL-1	148	302	800	340	>	700
KSL-7	149	303	355	315	530	330
LSB-37	150	306	320	50	240	170
Anubis-2	151	307	75	38	73	83
FLAK 17 CV	152	501	26	23	ND	ND
FLAK50 Q1V	153	502	64	92	ND	ND
D2A21V	154	503	150	210	ND	ND
FLAK 25 AM V	155	504	110	130	ND	ND
FLAK43 AM V	156	505	85	86	ND	ND
FLAK50D V	157	506	75	45	ND	ND
HECATE AM V	158	507	285	340	ND	ND
HECATE AC V	159	508	190	160	ND	ND
FLAK04 AM V	160	509	95	84	ND	ND
03 AM V	161	510	77	62	ND	ND
	162	67	4	7	ND	ND
	163	100	95	175	82	120
	164	69	101	45	63	66

Note: > indicates greater than 1000; ND indicates not determined; numbers are in μg/mL.

Table 5

Name	SEQ ID NO:	P No.	WI38	H1299	HeLaS3	PC3
HECATE AC	1	1	27	44	95	61
HECATE AM	2	2	66	140	50	44
SB37COOH	3	5	130	220	150	ND
SB-37 AM	5	12	950	720	>	630
SHIVA 10 AC	6	13	57	>	>	83
FLAK01 AM	8	23	34	64	82	41
FLAK03 AM	9	24	55	72	145	38
FLAK04 AM	10	25	24	37	20	12
FLAK05 AM	11	26	96	84	150	125
FLAK06 AM	12	27	37	16	25	8
FLAK06 AC	13	27B	101	54	80	16
FLAK06 AM	14	27C	520	170	260	280
KAL V	15	30	93	125	190	65
FLAK 17 AM	16	34	40	24	62	9
FLAK 26 AM	17	35	8	16	27	5
FLAK 25 AM	18	36	19	57	ND	19
HECATE 2DAc	19	37	80	150	ND	64
FLAK43 AM	20	38	12	33	35	10
FLAK44 AM	21	39	300	420	620	310
FLAK62 AM	22	40	>	>	>	435
FLAK 06R-AM	23	41	175	245	185	140
MSI-78 AM	24	42	67	150	ND	66
FLAK50	25	43	5	6	15	12
FLAK51	26	44	36	72	22	45
FLAK57	27	45	200	330	160	170
FLAK71	28	46	200	290	280	280
FLAK77	29	47	>	>	>	>
FLAK50V	30	48	41	17	44	32
FLAK50F	31	49	135	140	ND	77
FLAK26V AM	32	50	43	7	33	54
CAME-15	33	53	32	65	30	40
FLAK50C	34	54	97	80	190	90
FLAK50D	35	55	32	7	15	47
FLAK 50E	36	56	250	370	300	435
FLAK80	37	57	900	>	830	>
FLAK81	38	58	>	>	>	>
FLAK82	39	59	77	180	ND	81
FLAK83M	40	60	>	>	>	>
FLAK 26 Ac	41	61	93	127	170	66
INDOLICIDIN	42	63	ND	270	345	290
FLAK 17 C	43	64	37	30	30	46

FLAK 50H	44	65	320	450	210	470
FLAK 50G	45	66	240	130	140	170
SHIVA DERIV P69+KWKL	46	70	34	63	28	82
SHIVA 10 (1-18_ AC	47	71	355	320	570	270
SHIVA 10 PEPTIDE 71+KWKL	48	72	125	160	240	63
CA(1-7)Shiva10(1-16)	49	73	160	115	270	97
FLAK 54	50	74	335	670	260	660
FLAK 56	51	75	80	80	74	54
FLAK 58	52	76	445	860	380	675
FLAK 72	53	77	>	>	>	>
FLAK 75	54	79	>	>	>	>
SHIVA 10 (1-16) Ac	55	80	28	64	97	28
CA(1-7)Shiva10(1-16)-COOH	56	81	8	22	19	170
Indolocidin-ac	57	91	9	64	20	31
FLAK50B	58	92	43	25	670	83
FLAK50J	59	93	530	320	>	690
FLAK50I	60	94	6	ND	>	ND
FLAK50K	61	95	250	>	>	>
FLAK50L	62	96	>	>	>	>
Shiva-11	63	98	47	53	175	52
SHIVA 11 [(1-16)ME(2-9] -COOH	64	99	34	54	180	28
FLAK 50N	65	101	300	340	170	730
FLAK 50O	66	102	73	27	43	66
FLAK 50P	67	103	26	150	70	330
CA(1-&HECATE(11/23)	68	104	24	52	130	18
PYL-ME	69	105	430	>	>	ND
FLAG26-D1	70	106	>	920	700	>
VISHNU3	71	107	>	>	>	>
MELITTIIN	72	108	16	25	35	13
FLAK26-D2	73	109	>	>	>	>
FLAG26-D3	74	110	45	95	540	>
FLAK50 Q1	75	111	24	8	7	11
FLAK50 Q2	76	112	420	470	660	640
FLAK50 Q3	77	113	170	50	190	240

FLAK50 Q4	78	114	>	>	>	>
FLAK50 Q5	79	117	>	>	>	>
FLAK50 Q6	80	118	170	74	87	330
FLAK50 Q7	81	119	45	33	30	140
FLAK50 Q8	82	120	600	620	810	>
FLAK50 Q9	83	121	625	460	830	>
FLAK50 Q10	84	122	720	830	780	800
FLAK50 T1	85	123	600	>	940	>
FLAK50 T2	86	124	21	30	14	10
FLAK50 T3	87	125	90	76	220	145
FLAK50 T4	88	126	>	>	>	>
FLAK50 T5	89	127	760	770	610	>
FLAK90	90	128	500	>	700	>
FLAK91	91	129	>	790	550	>
FLAK92	92	130	>	>	>	>
FLAK93	93	131	>	>	>	>
FLAK50 Z1	94	132	>	>	>	>
FLAK50 Z2	95	133	>	>	>	>
FLAK50 Z3	96	134	>	>	>	>
FLAK50 Z4	97	135	110	115	215	310
FLAK50 Z5	98	136	>	450	400	900
FLAK50 Z6	99	137	>	>	>	>
FLAK50 Z7	100	138	>	>	>	>
FLAK50 Z8	101	139	550	850	>	>
FLAK50 Z9	102	140	>	>	285	>
FLAK94	103	141	420	>	>	ND
FLAK93B	104	142	73	115	55	60
FLAK50 Z10	105	143	>	>	>	>
FLAK96	106	144	750	225	275	350
FLAK97	107	145	>	>	240	>
FLAK98	108	146	270	93	640	440
FKRLA	109	147	83	93	>	340
FLAK91B	110	148	380	660	>	>
FLAK92B	111	149	>	>	>	>
FLAK99	112	150	125	185	320	74
FLAK50T6	113	151	>	>	>	>
FLAK50T7	114	152	620	410	>	>
FLAK95	115	153	130	50	140	97
FLAK50T8	116	154	600	400	>	640
FLAK50T9	117	155	>	>	>	ND
FLAK100-CO2H	118	156	230	ND	>	260
FAGVL	119	157	500	315	>	375
Modelin-5	120	159	82	74	275	145
Modelin-5-CO2H	121	160	700	470	550	450

FLAK120	126	165	470	56	400	340
FLAK121	127	166	>	>	>	>
FLAK96B	128	167	260	300	325	320
FLAK96G	129	168	>	>	>	>
FLAK96F	130	169	>	640	>	>
FLAK96C	131	170	>	>	>	>
FLAK96D	132	171	615	540	820	600
Modelin-8D	135	174	>	>	>	>
Modelin-8E	136	175	>	>	510	>
Flak 96H	137	176	>	>	>	>
Flak 96I	138	177	270	240	380	120
Flak 96J	139	178	405	>	>	>
Flak 96L	140	179	540	>	>	>
FLAK-120G	141	180	940	>	760	>
FLAK-120D	142	181	500	>	>	>
FLAK-120C	143	182	>	>	>	>
FLAK-120B	144	183	>	>	>	>
FLAK-120F	145	184	800	370	302	570
Magainin2wisc	146	300	52	60	125	45
D2A21	147	301	66	77	170	45
KSL-1	148	302	800	720	>	>
KSL-7	149	303	355	345	>	530
LSB-37	150	306	320	120	250	370
Anubis-2	151	307	75	160	100	66
	163	100	95	220	150	ND
	164	69	101	71	190	81

Note: > indicates greater than 1000; ND indicates not determined; numbers are in μg/mL.

It can be seen from Tables 4 and 5 that all targets challenged were inhibited by one or more of the peptides to an appreciable extent (i.e. LD50 less than 50 μg/ml). Table 6 below shows that 44 (29%) of the 150 peptides tested were active with some LD50 values at or below 50; 26 of the peptides were active on some targets at or below the LD50 value of 25; and 16 peptides were very active on one or more target strains with LD50 values at or below 10.

Table 7 below shows a broad spectrum of activity against six cancer cell types for various active peptides. It is noted that each target has one or more lead candidate peptides inhibitory to cell growth at an LD50 level of 10 or less.

Table 6: FLAK peptides showing substantial activity against cancer cell lines

LD50 values	Number of "active" peptides	Percent of 150 peptides tested
< or = 50 μg/ml	44	29%
< or = 25 μg/ml	26	17%
< or = 10 μg/ml	16	11%

Table 7: Activity and specificity of FLAK peptides against six cancer cell targets

LD50	Number of active peptides per target					
	MCF7 (breast)	SW480 (colon)	BMKC (melanoma)	H1299 (lung)	HeLaS3 (cervix)	PC3 (prostate)
< or = 50 μg/ml	31	25	19	19	17	20
< or = 25 μg/ml	17	13	8	10	8	11
< or = 10 μg/ml	6	5	3	4	1	5

Example 3: Stimulation and proliferation of leukocytes

In vitro viability of human leukocyte cells in the presence of different peptides at different concentrations was determined by an Alamar Blue protocol. Alamar Blue (Promega, Madison, WI) is an indicator dye, formulated to measure quantitatively the proliferation and cytotoxicity of the cells. The dye consists of an oxidation-reduction (redox) indicator that yields a colorimetric change and a fluorescent signal in response to cellular metabolic activity.

Assay protocol: Blood from a 50 year old male human was drawn and centrifuged at 1500 rpm for 15 minutes at room temperature. The buffy coat cells at the plasma-red blood cell interface were aspirated. Buffy coat cells (mainly lymphocyte cells) were then transferred into 15 ml centrifuge tubes containing 5 ml of RPMI-1640 medium+10% Fetal Bovine Serum (Gibco, Grand Island, NY). Additional medium was added to the tubes to bring the volume up to 10 ml. The buffy coat suspension was then carefully layered on 5 ml of Histopaque (Sigma Chemical Co., St. Louis, MO) and centrifuged at 1500 rpm for 30 minutes at room temperature. The interface which is mostly PBMCs (peripheral mononuclear cells) was aspirated and transferred to a 15 ml conical centrifuge tube and, resuspended in 2 ml cold RPMI-1640 and brought up to 15 ml with cold RPMI-1640 medium. Cells were centrifuged at 1500 rpm for 10 minutes. The supernatant was then aspirated and discarded. The cell pellet was re-suspended in 1 ml of cold RPMI

1640 and brought up to 15 ml with RPMI medium. This step was repeated twice, except that in the last step, the cells were resuspended with 1 ml of cold RPMI-1640 medium and cell counts were performed with a hemocytometer according to the Sigma cell culture catalogue.

Pokewood mitogen was used as a control along with positive and negative controls. Negative control cells were killed with 70% methanol. Positive (+) control cells were incubated in RPMI medium (untreated). 20 ml of AlamarBlue was added to the cells, and readings were taken after 24 hours, 48 hours, 72 hours, and 96 hours using a fluorimeter (excitation 544/transmission 590 nm).

Calculations were performed using the following formula:

$$\% \text{ treated cell viability} = \frac{\text{Peptide treated sample (adj. for negative control)}}{\text{Positive control (adj. for negative control)}} \times 100\%$$

Using the protocol described immediately above, about 100-150 peptides were screened for their stimulatory and/or inhibitory actions upon the growth of human leukocyte ("WBC") cells as compared to the growth of untreated positive control cells. The data in Table 8 below show that various selected FLAK peptides are stimulatory at low concentrations (0.1 to 1.0 μg/ml), whereas certain of the peptides become inhibitory (causing cell death) at higher concentrations. Several of the peptides (i.e. SEQ ID NOS: 5, 143, and 160) are stimulatory (and/or proliferative) at all concentrations through 500 μg/ml.

The Alamar Blue stain used in the protocol permeates both cell and nuclear membranes, and is metabolized in the mitochondria to cause the change in color. The resulting fluorometric response is therefore a result of total mitochondrial activity caused by cell stimulation and/or mitosis (cell proliferation). The increase in values (for treated cells, as a percent of values for untreated cells) with increased incubation time (120 hours vs. 48 hours) may be attributed to increased cell proliferation in addition to stimulation of cell metabolic activity caused by the peptide

Table 8 presents viability data, as percent of untreated positive control, for human leukocytes (white blood cells, "WBC") in the presence of selected FLAK peptides. The

table also shows for each of these peptides its toxicity (LD50 values) to human red blood cells (RBC) and to human fibroblast cells (WI38). Those certain peptides which are stimulatory to WBCs at low peptide concentrations (i.e. 10 μg/ml or less) and are inhibitory or toxic to WBCs at higher concentrations are also relatively more toxic to RBCs and to fibroblasts than those peptides which are stimulatory and not inhibitory to WBC growth even at concentrations as high as 500 μg/ml.

In limited experiments with other than the Alamar Blue protocol described above, it has been qualitatively determined that those peptides which cause stimulation and proliferation of leukocytes are active upon both the phagocytic and lyphocyte cell components of the mammalian lymphatic system. As such, certain of the stimulatory FLAK peptides which are relatively non-toxic to mammalian cells at therapeutic dose levels may be used as immunomodulators to treat humans or other mammals with compromised immune systems. Such treatment may be administered systemically *in vivo* or by extra-corporeal treatment of whole blood or blood components to be reinfused to the donor. Such therapy would serve to counteract immune deficiency in neutropenic patients caused by age, disease, or chemotherapy and would stimulate natural immune responses to prevent or combat pathogenic infections and growth of certain cancer cell lines or to enhance wound healing processes involving the lymphoid system.Table 9 is a more detailed example (with one peptide, SEQ ID NO:10) of the phenomenon showing the relationships of concentration and time as they effect stimulation, proliferation, and inhibition of the leukocytes.

Table 8: Human leukocyte (WBC) stimulation / proliferation & inhibition by selected FLAK peptides

	Peptide conc.	0.1 μg/ml	0.1 μg/ml	1 μg/ml	1 μg/ml	10 μg/ml	10 μg/ml
SEQ ID NO:	P Number	48 hours	120 hours	48 hours	120 hours	48 hours	120 hours
5	12	111	124	115	136	118	141
10	25	117	135	104	118	99	119
12	27	108	117	110	126	99	114
17	35	115	113	119	105	114	81
20	38	115	110	119	117	114	109
25	43	115	100	119	114	114	104

58	92	112	120	112	114	98	99
66	102	100	89	102	90	97	110
143	182	101	134	96	117	101	133
150	306	97	94	101	113	94	109

SEQ ID NO:	Peptide conc. P Number	100 µg/ml 48 hours	100 µg/ml 120 hours	500 µg/ml 48 hours	500 µg/ml 120 hours	RBC toxicity LD50	WI-38 toxicity LD50
5	12	116	151	101	119	>1000	950
10	25	27	43	27	45	60	24
12	27	30	43	23	39	125	37
17	35	73	42	72	43	200	8
20	38	73	60	72	57	350	12
25	43	73	39	72	37	20	5
58	92	35	30	26	26	300	125
66	102	37	32	17	15	300	73
143	182	109	150	105	132	>1000	660
150	306	109	140	112	140	>1000	320

Table 9: Human leukocyte (WBC) stimulation / proliferation and inhibition by FLAK peptide SEQ ID NO:10 (P25)

Time of incubation	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	500 µg/ml
24 hours	111	98	88	10	10
48 hours	117	104	99	27	27
72 hours	119	105	102	31	32
96 hours	128	112	110	38	40
120 hours	135	118	119	43	45

Note: Number values are percent cell viability relative to control cells.

Example 4: Stimulation and proliferation of fibroblasts

The cyQUANT cell proliferation assay provides a convenient, rapid and sensitive procedure for determining the density of cells in culture. The assay has a linear detection range extending from 50 or fewer to at least 50,000 cells in 200 µl volumes using a single dye concentration. The assay is ideal for cell proliferation studies as well as for routine cell counts and can be used to monitor the adherence of cells to surfaces.

Procedure: Different cell lines were maintained with different medium according to the ATCC. Cells were trypsinized with 8 ml of Trypsin (0.25%, Fisher, Pittsburgh, PA). The cell suspension was centrifuged for 10 minutes at 100 rpm. The supernatant was removed and discarded without disturbing the cell pellet. A concentrated cell suspension was prepared in 1.0 ml of medium to obtain a density of about 10^5 to 10^6 cells/ml. The actual cell density was determined by counting the cells using a hemocytometer with the Trypan Blue method. Cell numbers were adjusted to obtain equal number of cells per 200 µl volume. Cells were plated with 0% FBS, 2.5% FBS, 5% FBS and 10% FBS. The plates were incubated at 37 °C for a time sufficient to allow the cells to attach. For long-term proliferation studies, 100 µl of medium was removed from each well each day and replaced with fresh medium.

At the desired time, the medium was removed from the adherent cells in a 96 well plate. These cells were already treated with test agents. The cells were frozen in the plate at -70 °C for 30 minutes. The cells were thawed at room temperature. CyQuant GR dry/Cell Lysis Buffer (200 µl) was added to each sample cell. The cells were incubated at room temperature for 15 minutes while protected from the light. Fluorescence was measured using fmax at 485-538 nm.

The above CyQuant protocol was used to examine possible peptide stimulation of fibroblasts. In the following Table 10, data are shown for selected peptides demonstrating their effect on human fibroblast cells (WI38). In the table, the substantial stimulatory and/or proliferative property of selected peptides, as a function of concentration is evident. The values are viability of treated cells expressed as percent (%) above or below positive control (untreated cells). Table 11 shows that the fibroblast stimulation and/or proliferation effect is enhanced for certain peptides in the presence of other growth factors. This is shown by the addition of Fetal Bovine Serum (FBS) to the medium. Negative values indicate inhibitory action of the peptide, especially at concentrations above 10 µg/ml.

Table 10: Human fibroblast (WI-38) cell stimulation by selected FLAK peptides

SEQ ID NO:	P Number	% FBS in serum	Peptide concentration 0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml
2	2	0	-27	-3	27	-82
		2.5	26	57	23	-66
4	11	0	19	34	50	-40
		2.5	50	52	62	14
6	13	0	76	68	93	95
8	23	0	21	78	10	-48
		2.5	16	23	58	75
10	25	0	50	59	29	-27
14	27C	0	60	85	90	63
15	30	0	60	75	20	35
17	35	0	45	70	65	50
20	38	0	44	22	75	53
35	55	0	1	12	30	76
5	12	0 (24h inc)	93	90	116	65
58	92	0 (24h inc)	109	114	132	36
71	107	0	18	27	26	24
80	118	0	12	-4	-7	-1
		0 (24h inc)	24	55	48	24
		3	61	70	68	72
126	165	0	51	77	115	50

Note: Number values are percent cell viability above or below control. Incubations were 48 hours unless otherwise indicated. SEQ ID NOS:5 and 71 are not FLAK peptides.

Table 11: Effect of growth factors on human fibroblast (WI38) cell stimulation

SEQ ID NO:	P Number	% FBS in serum	Peptide concentration 0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml
2	2	0	-27	-3	27	-82
		2.5	26	57	23	-66
4	11	0	19	34	50	-40
		2.5	50	52	62	14
8	23	0	21	78	10	-48
		2.5	16	23	58	75
80	118	0	12	-4	-7	-1
		3	61	70	68	72

Note: Number values are percent cell viability above or below control.

Example 5: Toxicity assay - Red blood cell (RBC) hemolysis, and leukocyte (WBC) and fibroblast (WI38) inhibition

Table 12 below summarizes the RBC, WBC, and WI38 toxicity data for typical FLAK peptides. The three RBC, WBC, and WI38 values (LD50) are generally consistent directional indicators of peptide toxicity. In choosing a peptide for possible treatment of a given indication it is important to match the therapeutic activity and specificity of the peptide with its possible toxic properties. The SEQ ID NO:5 peptide is not a FLAK peptide, but rather it is SB-37, a close homolog of Cecropin B. It has previously been shown not to be as active as the FLAK peptides as an antibacterial agent, but to possess wound healing properties as demonstrated *in vivo* in a rat model. This probably results from its stimulatory and proliferative effects on both mammalian leukocytes and fibroblasts.

The protocols for WBC and WI38 stimulation have been discussed above. The RBC protocol follows Table 12.

Table 12: *In vitro* toxicity of selected FLAK peptides on red blood cells (RBC), human leukocytes (WBC), and human fibroblasts (WI38)

SEQ ID NO:	P Number	RBC LD50 µg/ml	WBC LD50 µg/ml	WI38 LD50 µg/ml
5	12	>1000	>500	60
10	25	60	79	60
11	26	900	185	100
12	27	125	78	60
16	34	200	77	200
17	35	200	64	25
20	38	350	160	100
25	43	20	70	25
30	48	130	78	70
35	55	30	80	28
58	92	300	51	400
66	102	300	115	45

The RBC protocol is as follows. Well positions of each dilution and untreated controls are recorded on the lid of a 96-well plate. When the cells were confluent, the media is removed, and replaced with freshly prepared sample dilutions to a final volume of 200 µl. Test agent was added into designed wells of the 96-well plate. The 200 µl

fresh medium was added to positive control wells; and 200 µl of 70% ethanol was added to negative control wells. The plate was incubated overnight at 37 °C, 5% CO_2, and at least 90% humidity. Room temperature AlamarBlue solution (20 µl) was added to all wells. The plates were read spectrofluorometrically (excitation 544 nm, emission 590 nm). The plates were incubated for 3 hours at 37 °C, 5% CO_2, and at least 90% humidity. The plates were read again at 3 and 24 hours incubation. The LD50 endpoint was determined from the graph by reading from where the 50 percent point intercepts the Dose Response Curve to the concentration along the x-axis. That concentration is the LD50 value. The LD50 value for test agents within a single test agent class can be used to rank-order their relative toxicities or to correlate with *in vivo* data.

This hemolytic assay is based upon that presented in *Journal of Peptide Research* 53: 82-90 (1999). Preparation of all media, stock solutions and dilutions were performed in a laminar flow hood to minimize or prevent contamination. All procedures were performed according to safety protocols pertaining to the handling and disposal of human body fluids.

Red blood cells (RBCs) were washed three times with PBS (35 mM phosphate buffer 0.15 M NaCl, pH 7.0). RBCs suspended in PBS (0.4% (v/v); about 10 ml per 15 peptides) were prepared. Suspensions (100 µl) were aliquoted to each sample and control tube. Serially diluted peptide solutions (100 µl) were pipetted into the sample tubes. Negative control tubes contained 100 µl PBS; positive control tubes contained 100 µl 1% Triton-X100 detergent. All tubes were incubated for 1 hour at 37 °C. The tubes were removed from the incubator and centrifuged at 1000g for 5 minutes. Supernatant (100 µl) was pipetted to a 96-well polyvinyl chloride plate. The absorbance at 414 nm (A_{414}) was measured, and used to calculate the percent hemolysis according to the following formula.

$$\frac{(A_{414} \text{ in peptide solution} - A_{414} \text{ in PBS})}{(A_{414} \text{ in Triton-X 100} - A_{414} \text{ in PBS})} \times 100\%$$

Percent hemolysis is plotted against peptide concentration, and the concentration at which 50% hemolysis is determined (LD_{50}). The following Table 13 details the results of the hemolytic assay using the peptides discussed herein.

Table 13

Peptide name	SEQ ID NO:	P Number	LD_{50} µg/mL
Hecate AC #1010	1	1	100
Hecate AM	2	2	10
SB-37 AC #1018	3	5	>
Shiva 10 AM	4	11	76
SB-37 AM	5	12	>
Shiva 10 AC #1015	6	13	50
Magainin 2	7	16	550
FLAK01 AM	8	23	300
FLAK03 AM	9	24	10
FLAK04 AM	10	25	16
FLAK05 AM	11	26	90
FLAK06 AM	12	27	125
FLAK06 AC	13	27B	700
FLAK06 R-AC	14	27C	250
KALV	15	30	150
FLAK 17 AM	16	34	200
FLAK 26 AM	17	35	200
FLAK 25 AM	18	36	85
Hecate 2DAc	19	37	30
FLAK43 AM	20	38	350
FLAK44 AM	21	39	>
FLAK62 AM	22	40	>
FLAK 06R-AM	23	41	40
MSI-78 AM	24	42	300
FLAK50	25	43	20
FLAK51	26	44	90
FLAK57	27	45	700
FLAK71	28	46	900
FLAK77	29	47	>
FLAK50V	30	48	200
FLAK50F	31	49	225
FLAK26V AM	32	50	420
CAME-15	33	53	20
FLAK50C	34	54	250
FLAK50D	35	55	20
FLAK 50E	36	56	600
FLAK80	37	57	>

FLAK81	38	58	>
FLAK82	39	59	1000
FLAK83M	40	60	>
FLAK 26 Ac	41	61	390
Indolicidin	42	63	375
FLAK 17 C	43	64	6
FLAK 50H	44	65	950
FLAK 50G	45	66	600
Shiva deriv P69+KWKL	46	70	80
Shiva 10 (1-18_AC	47	71	>
Shiva 10 peptide 71+KWKL	48	72	110
CA(1-7)Shiva10(1-16)	49	73	90
FLAK 54	50	74	>
FLAK 56	51	75	750
FLAK 58	52	76	>
FLAK 72	53	77	>
FLAK 75	54	79	>
Shiva 10 (1-16) Ac	55	80	900
CA(1-7)Shiva10(1-16)-COOH	56	81	8
Indolocidin-ac	57	91	40
FLAK50B	58	92	300
FLAK50J	59	93	>
FLAK50I	60	94	350
FLAK50K	61	95	>
FLAK50L	62	96	>
Shiva-11	63	98	60
Shiva 11[(1-16)ME(2-9)]-COOH	64	99	25
FLAK 50N	65	101	550
FLAK 50O	66	102	500
FLAK 50P	67	103	650
CA(1-&Hecate(11/23)	68	104	70
PYL-ME	69	105	ND
FLAG26-D1	70	106	>
Vishnu3	71	107	>
Melittin	72	108	<1
FLAK26-D2	73	109	>
FLAG26-D3	74	110	>
FLAK50 Q1	75	111	60
FLAK50 Q2	76	112	>
FLAK50 Q3	77	113	1000
FLAK50 Q4	78	114	>
FLAK50 Q5	79	117	>
FLAK50 Q6	80	118	700

FLAK50 Q7	81	119	400
FLAK50 Q8	82	120	>
FLAK50 Q9	83	121	>
FLAK50 Q10	84	122	>
FLAK50 T1	85	123	1000
FLAK50 T2	86	124	55
FLAK50 T3	87	125	>
FLAK50 T4	88	126	>
FLAK50 T5	89	127	>
FLAK90	90	128	>
FLAK91	91	129	>
FLAK92	92	130	>
FLAK93	93	131	>
FLAK50 Z1	94	132	>
FLAK50 Z2	95	133	>
FLAK50 Z3	96	134	>
FLAK50 Z4	97	135	900
FLAK50 Z5	98	136	>
FLAK50 Z6	99	137	>
FLAK50 Z7	100	138	20
FLAK50 Z8	101	139	>
FLAK50 Z9	102	140	>
FLAK94	103	141	900
FLAK93B	104	142	900
FLAK50 Z10	105	143	>
FLAK96	106	144	600
FLAK97	107	145	>
FLAK98	108	146	180
FKRLA	109	147	300
FLAK91B	110	148	>
FLAK92B	111	149	>
FLAK99	112	150	650
FLAK50T6	113	151	>
FLAK50T7	114	152	880
FLAK95	115	153	800
FLAK50T8	116	154	450
FLAK50T9	117	155	>
FLAK100-CO2H	118	156	10
FAGVL	119	157	850
Modelin-5	120	159	ND
Modelin-5-CO2H	121	160	>
FLAK120	126	165	350
FLAK121	127	166	>
FLAK96B	128	167	200

FLAK96G	129	168	600
FLAK96F	130	169	>
FLAK96C	131	170	>
FLAK96D	132	171	550
Modelin-8D	135	174	>
Modelin-8E	136	175	>
Flak 96	137	176	>
Flak 96I	138	177	400
Flak 96J	139	178	>
Flak 96L	140	179	850
FLAK-120G	141	180	>
FLAK-120D	142	181	>
FLAK-120C	143	182	>
FLAK-120B	144	183	>
FLAK-120F	145	184	850
Magainin2wisc	146	300	250
D2A21	147	301	10
KSL-1	148	302	>
KSL-7	149	303	500
LSB-37	150	306	>
Anubis-2	151	307	>
FLAK17CV	152	501	15
FLAK50Q1V	153	502	100
D2A21V	154	503	20
FLAK25AMV	155	504	70
FLAK43AMV	156	505	620
FLAK50DV	157	506	120
HECATE AMV	158	507	20
HECATE ACV	159	508	70
FLAK04AMV	160	509	40
FLAK03AMV	161	510	10
D-Shiva 10 AC	162	67	40
Shiva 11 AC	163	100	>
Shiva 10 (1-18)AM	164	69	900

Note: > indicates greater than 1000; ND = not determined.

Example 6: Effects of valine substitution

Changing a peptide sequence where the first amino acid is valine, and particularly when the first amino acid is changed from phenylalanine to valine, can lead to desirable properties. The red blood cell and fibroblast cell (WI38) toxicity can be decreased, while not significantly decreasing other desirable properties. Table 14 below shows numerous

examples (14) of reducing the indicated toxicity of a peptide as seen from increase in viability of both red blood cells and fibroblast cells when treated with peptide. LD50 values are in µg/ml.

Table 14

SEQ. ID NO:	P No.	Sequence	Hemolysis RBC LD50	WI-38 LD50
2	2	FALALKALKKALKKLKKALKKAL-NH2	12	66
15	30	VALALKALKKALKKLKKALKKAL-NH2	150	93
17	35	FAKKLAKLAKKLAKLAL-NH2	150	25
32	50	VAKKLAKLAKKLAKLAL-NH2	420	45
25	43	FAKLLAKLAKKLL-NH2	20	25
30	48	VAKLLAKLAKKLL-NH2	130	160
86	124	FAKLLAKLAKKVL-NH2	55	21
116	154	VAKLLAKLAKKVL-NH2	870	110
126	165	FALALKALKKL-NH2	350	850
141	180	VALALKALKKL-NH2	850	1000
43	64	FAKALKALLKALKAL-NH2	6	37
152	501	VAKALKALLKALKAL-NH2	15	26
75	111	FAKFLAKFLKKAL-NH2	5	25
153	502	VAKFLAKFLKKAL-NH2	100	64
147	301	FAKKFAKKFKKFAKKFAKFAFAF-NH2	10	66
154	503	VAKKFAKKFKKFAKKFAKFAFAF-NH2	20	150
18	36	FAKKLAKLAKKLAKLALAL-NH2	12	19
155	504	VAKKLAKLAKKLAKLALAL-NH2	70	110
20	38	FAKKLAKLAKKLLAL-NH2	350	100
156	505	VAKKLAKLAKKLLAL-NH2	620	85
35	55	FAKLLAKALKKLL-NH2	20	32
157	506	VAKLLAKALKKLL-NH2	120	75
1	1	FALALKALKKALKKLKKALKKAL-COOH	20	27
159	508	VALALKALKKALKKLKKALKKAL-COOH	70	190

10	25	FALALKALKKLAKKLKKLAKKAL-NH2	16	24
160	509	VALALKALKKLAKKLKKLAKKAL-NH2	40	95
9	24	FALALKALKKLLKKLKKLAKKAL-NH2	10	55
161	510	VALALKALKKLLKKLKKLAKKAL-NH2	10	77

Although the effects of reduction of toxicity to mammalian cells by valine substitution is accompanied by modest reductions of therapeutic activity against microbial pathogens and cancer cells, there are some cases in which the valine substitution results in a desirable increase in therapeutic activity. This can be seen in the following Table 15 where it is shown that the valine substitution in some cases has increased the peptide's activity against the gram negative bacterium *Pseudomonas*.

Hemolysis and WI38 values represent LD50 values. P. aerug values represent MIC values in μg/mL against *Pseudomonas aeruginosa* ATCC accession number 9027.

Table 15

SEQ ID NO:	P No.	Sequence	Hemolysis	WI38	P. aerug
17	35	FAKKLAKLAKKLAKLAL	100	25	200
32	50	VAKKLAKLAKKLAKLAL	420	45	15
25	43	FAKLLAKLAKKLL	20	25	100
30	48	VAKLLAKLAKKLL	200	160	5
86	124	FAKLLAKLAKKVL	300	21	100
116	154	VAKLLAKLAKKVL	450	110	100

Example 7: Preferred peptides

Preferred peptides can be selected from the above described experimental data. Preferred antimicrobial peptides for gram positive or gram negative bacteria can be selected as having MIC values of less than or equal to about 10 μg/ml, or as having MBC values of less than or equal to about 25 μg/ml. Preferred antifungal peptides can be selected as having MIC or MBC values of less than or equal to about 25 μg/ml. Preferred anticancer peptides can be selected as having LD50 values of less than or equal to about 25 μg/ml.

The following Table 16 lists representative preferred peptides, where an 'X' indicates that the peptide is a preferred peptide for that column's property. The peptide's "length" is the number of amino acid residues in the sequence.

Table 16

SEQ ID NO:	P-number	Length (AA)	Anti-bacterial	Anti-fungal	Anti-cancer
1	1	23	X		X
2	2	23	X	X	X
4	11	23	X		
6	13	23	X		
8	23	23	X		X
10	25	23	X	X	
11	26	21	X	X	X
12	27	19	X	X	
13	27B	19	X	X	X
14	27C	19	X		
15	30	23	X		
16	34	16	X	X	X
17	35	17	X	X	X
18	36	19	X		X
19	37	23	X		X
20	38	15	X		X
23	41	19	X		
25	43	13	X	X	X
26	44	15	X		X
27	45	14	X		
28	46	15	X		
29	47	12			X
30	48	13	X	X	X
31	49	12	X		
32	50	17	X		X
34	54	13	X		
35	55	13	X	X	X
36	56	13	X		
41	61	15	X		
43	64	15	X		
45	66	13	X		
46	70	23	X		X
50	74	13	X		
51	75	13	X		X
52	76	14	X		

55	80	23	X		X
56	81	23	X		X
57	91	15	X	X	X
58	92	13	X		X
60	94	13	X		
65	101	13	X	X	
66	102	13	X	X	
67	103	12	X		X
68	104	20	X		
74	110	12	X		X
75	111	13	X		
77	113	13	X	X	
80	118	13	X	X	
81	119	14	X	X	
84	122	13		X	
85	123	10	X	X	X
86	124	13	X		
87	125	13	X		
93	131	5	X	X	
106	144	12	X	X	
108	146	13	X		
112	150	17	X	X	
115	153	17		X	
116	154	13	X	X	
126	165	11	X	X	
128	167	12		X	
131	170	10		X	
143	182	10			X
152	501	15	X		X
162	67	23	X	X	
163	100	13	X		
164	69	23	X	X	
165	97	13			

Preferred peptides for stimulation and proliferation can also be selected. The following Table 17 lists representative preferred peptides, where an 'X' indicates that the peptide is a preferred peptide for that column's property. Peptides which are stimulatory for leukocytes at 0.1 μg/ml to 1.0 μg/ml concentration are preferred, as at this concentration the peptides are not toxic to red blood cells, WI-38 fibroblasts, or to human leukocytes. Peptides which are stimulatory for fibroblasts at 0.1 μg/ml to 1.0 μg/ml are preferred, as at this concentration the peptides are not toxic.

Table 17: Preferred peptides for leukocyte and fibroblast stimulation / proliferation

SEQ ID NO:	P-number	Length	Leukocyte	Fibroblast
1	29	23	X	X
2	2	23	X	X
5	12	38	X	X
6	13	23	X	X
8	23	23	X	X
10	25	23	X	X
11	26	21	X	X
12	27	19	X	X
13	27B	19	X	X
14	27C	19	X	X
15	30	23	X	X
16	34	16	X	X
17	35	17		X
20	38	15		X
27	45	14		X
28	46	15		X
30	48	13		X
32	50	17	X	
34	54	13	X	X
45	66	13	X	X
46	70	23	X	X
50	74	13	X	X
51	75	13		X
55	80	23		X
56	81	23	X	X
57	91	15	X	X
58	92	13		X
59	93	13		X
60	94	13	X	X
61	95	13		X
65	101	13		X
66	102	13	X	X
71	107	19		X
74	110	12		X
75	111	13		X
77	113	13		X
80	118	13		X
81	119	14		X
87	125	13	X	X
90	128	5	X	X

91	129	5		X
92	130	5		X
115	153	17		X
116	154	13	X	
126	165	11		X
127	166	11		X
129	168	6	X	X
132	171	11		X
137	176	11	X	
138	177	12	X	
139	178	11	X	X
140	179	11	X	X
141	180	11	X	X
142	181	10	X	X
143	182	10	X	X
144	183	5	X	X
145	184	5	X	X
159	508	23	X	X
162	67	23	X	X
164	69	18		X

Example 8: Synergistic effects with lysozyme

Synergy between lytic peptides and lysozyme was assayed. Sterilized milk was inoculated with bacteria to 5 x 10^5 per ml. Peptide Shiva-10 (SEQ ID NO:4) was added to 10 μg/ml, and chicken lysozyme was added to 1 mg/ml. The percent killing of bacteria was determined.

Table 18

	Staph. aureus	*Pseud. aeruginosa*
Peptide and lysozyme	0%	100%
Peptide	0%	0%
Lysozyme	0%	0%

Synergy between cecropin SB-37 (SEQ ID NO:5) and lysozyme was determined against *Pseudomonas syringae* pv. *tabaci* (PSPT), *Pseudomonas solanacearum* (PS), *Erwinia caratovora subsp. carotova* (EC), and *Xanthomonas campestris* pv. *campestris* (XC). LD_{50} (μM) values were determined.

Table 19

	SB-37	Lysozyme	SB-37 and Lysozyme
PSPT	5.20	>	0.19
PS	64.0	>	16.0
EC	1.48	>	0.44
XC	0.57	>	0.027

> indicates greater than 1000.

Synergy between Shiva-1 and lysozyme was determined. The percent viability of *Pseudomonas aeruginosa* was determined relative to blank controls. Lysozyme was used at the same molar concentration as the peptide.

Table 20

Peptide concentration (μM)	SB-37	Shiva-1	Lysozyme (1x)	Shiva-1 and Lysozyme (1x)
0	100	100	100	100
0.01	100	100	100	56.6
0.1	79.4	69.6	82.2	25.8
1	48.8	37.9	52.1	4.4
5	38.5	1.5	7.9	0.2
7.5	0.7	0.1	0.6	0
25	0	0	0.4	0

Synergy between Shiva-1 and lysozyme was determined. The percent viability of gram positive *S. intermedius* 19930, *S. intermedius* 20034, and *S. aureus* was determined relative to blank controls. Lysozyme was used at ten times the molar concentration as the peptide.

Table 21: *S. intermedius* 19930

Peptide concentration (μM)	SB-37	Shiva-1	Lysozyme (10x)	Shiva-1 and Lysozyme (10x)
0	100	100	100	100
0.01	100	100	100	100
0.1	94.7	81.8	100	79.2
0.5	69.4	65.0	81.3	65.1
1	42.5	42.1	53	43
5	36.1	35.2	49.5	17.2
10	5.6	1.2	34.4	1.1
50	0	0	22	0

Table 22: *S. intermedius* 20034

Peptide concentration (μM)	SB-37	Shiva-1	Lysozyme (10x)	Shiva-1 and Lysozyme (10x)
0	100	100	100	100
0.01	100	100	100	100
0.25	85.4	87.1	100	85.1
0.5	68.0	80.0	59.0	53.4
0.75	62.2	60.1	42.3	41.0
5	35.1	4.1	38.3	4.3
50	0	0	10.0	0

Table 23: *S. aureus*

Peptide concentration (μM)	SB-37	Shiva-1	Lysozyme (10x)	Shiva-1 and Lysozyme (10x)
0	100	100	100	100
0.01	100	100	100	100
0.1	100	100	100	100
0.5	81.0	50.1	100	100
1	47.5	24.4	51.0	31.2
5	31.8	15.9	18.4	8.2
10	5.6	4.5	13.3	4.5
50	1.9	1.6	9.5	1.4

Synergy experiments can also be performed using peptides in the presence of EDTA, which potentiates the peptides additively or synergistically.

Example 9: Synergistic effects with antibiotics

Synergy between peptide Shiva-10 (SEQ ID NO:4) and various antimicrobial agents was investigated against *Escherichia coli* 25922. The following table illustrates the beneficial effects of combining the peptide with the agents, where the numbers are the minimum bactericidal concentration (MBC; μg/mL).

Table 24

Agent	Without peptide	With peptide
Shiva-10	50	n/a
Ticarcillin	100	50 (15 μg/mL peptide)
Cefoperazone	150	2.5 (15 μg/mL peptide)
Doxycycline	5	1 (15 μg/mL peptide)
Neomycin	100	5 (5 μg/mL peptide)

Amikacin	150	50 (5 μg/mL peptide)
Tetracycline	10	2.5 (5 μg/mL peptide)

Synergy between peptide Shiva-10 (SEQ ID NO:4) and various antimicrobial agents was investigated against *Staph. aureus* 29213. The following table illustrates the beneficial effects of combining the peptide with the agents, where the numbers are the minimum bactericidal concentration (MBC; μg/mL).

Table 25

Agent	Without peptide	With 5 μg/mL peptide
Shiva-10	200	n/a
Ampicillin	5	2.5
Ticarcillin	25	15
Cefoperazone	10	2.5
Tobramycin	25	10
Tetracycline	10	1

Synergy between peptide FLAK 26AM (P35; SEQ ID NO:17) and various antimicrobial agents was investigated against *Staph. aureus* 29213 MBC. The following table illustrates the beneficial effects of combining the peptide with the agents, where the numbers are the minimum bactericidal concentration (MBC; μg/mL). This experiment determined the peptide MBC in the absence of the antimicrobial agent, or in the presence of the indicated concentration of antimicrobial agent

Table 26

Agent	MBC of peptide
FLAK 26AM alone	50
Vancomycin (1 ppm)	32
Cefoperazone (0.25 ppm)	20

Synergy between doxacycline and various peptides was investigated against *P. aeruginosa* 27853. The following table illustrates the beneficial effects of combining doxacycline and the peptides, where the numbers are the minimum bactericidal concentration (MBC; μg/mL). When combined with the peptides, the doxacycline was held at 10 ppm concentration.

Table 27

Agent	Without doxacycline	With doxacycline
Doxacycline	n/a	100
SB-37 (P5; SEQ ID NO:3)	200	30
FLAK 26AM (P35; SEQ ID NO:17)	50	32

Synergy between tetracycline and various peptides was investigated against *Escherichia coli* 25922 MBC. The following table illustrates the beneficial effects of combining tetracycline and the peptides, where the numbers are the minimum bactericidal concentration (MBC; μg/mL). When combined with the peptides, the concentration of tetracycline was held at 1.5 ppm.

Table 28

Agent	Without tetracycline	With tetracycline
Tetracycline	n/a	10
FLAK 06AM (P27; SEQ ID NO:12)	75	25
FLAK 26AM (P35; SEQ ID NO:17)	50	20

Example 10: Synergistic effects with chemotherapy agents

Other investigators have reported that lytic peptides which are inhibitory to cancer cells will act synergistically with conventional cancer chemotherapy drugs. The FLAK peptides are no exception. Table 29 below demonstrates for example that selected FLAK peptides are synergistic with Tamoxifen in the inhibition of the MCF7 line of breast cancer cells. Table 30 lists other more active anti-cancer peptide candidates for synergistic application with Tamoxifen or other cancer therapy drugs.

Tables 29 and 30 also show toxicity of the selected peptides against RBCs, WBCs, and WI38 cells. When used at very low non-toxic levels selected anti-cancer peptides can synergistically potentiate other chemotherapy agents to permit their effective use at substantially lower dose levels with consequently fewer side effects.

Table 29: Synergy of FLAK peptides with tamoxifen on MCF7 cells

		Active agent	LD50 on MCF7 cells		
SEQ ID NO: (P No.)	Agent	MCF7 LD50 μg/ml	Peptide conc. μg/ml	Tamox. conc. μg/ml	Total conc. μg/ml
	Tamoxifen	20	0	20	20

164 (69)	Alone With Tamox.	79	2.5	4.6	7.1
145 (184)	Alone With Tamox.	240	10	4	14
121 (160)	Alone With Tamox.	240	11	3.7	14.7
106 (144)	Alone With Tamox.	310	35	7.7	42.7

SEQ ID NO: (P No.)	MCF7 LD50 µg/ml	RBC LD50 µg/ml	WI38 LD50 µg/ml	WBC LD50 µg/ml
164 (69)	79	900	60	140
145 (184)	240	850	1000	410
121 (160)	240	> 1000	700	900
106 (144)	310	600	740	320
17 (35)	9	200	25	25
32 (50)	32	420	40	420
20 (38)	17	350	100	54

Table 30: Other highly active peptide candidates for synergistic anti-cancer applications

SEQ ID NO: (P No.)	MCF7 LD50 µg/ml	RBC LD50 µg/ml	WI38 LD50 µg/ml	WBC LD50 µg/ml
17 (35)	9	200	25	25
32 (50)	32	420	40	420
20 (38)	17	350	100	54

Example 11: Synergistic effects with growth factors

It has been shown above in Example 4 and Table 11 that certain of the FLAK peptides are synergistic with other mitogens or growth factors in the stimulatory and/or proliferative properties of the peptides.

Example 12: Activity against drug resistant strains

Peptides were assayed for their activity against tobramycin sensitive and resistant strains. As shown in the following Table 31, peptides P56 (SEQ ID NO:36), P74 (SEQ ID NO:50), and P125 (SEQ ID NO:87) showed enhanced activity against tobramycin resistant (tr) *Pseudomonas* ATCC 13096 than against tobramycin sensitive (ts)

Pseudomonas ATCC 27853. The same three peptides showed enhanced activity against clinical tobramycin resistant strain 960890198-3c (Table 31).

Table 31

Peptide	tr *Pseudomonas* 13096	ts *Pseudomonas* 27853
SEQ ID NO:36 (P56)	16	125
SEQ ID NO:50 (P74)	16	125
SEQ ID NO:87 (P125)	4	31

Table 32

Peptide	tr *Pseudomonas* 960890198-3c	ts *Pseudomonas* 27853
SEQ ID NO:36 (P56)	> 50	125
SEQ ID NO:50 (P74)	25	125
SEQ ID NO:87 (P92)	50	63

Example 13: Wound healing

The inventive peptides can be used in compositions for topical or systemic delivery in wound healing applications. The compositions can be a liquid, cream, paste, or other pharmaceutically acceptable formulation. The compositions may contain other biologically active agents. The compositions may contain pharmaceutically acceptable carriers.

Those peptides preferred for wound healing, shown in Table 33 below, are peptides which were preferred for either, or or both, leukocyte or fibroblast stimulation.

Table 33: Preferred peptides for wound healing

SEQ ID NO:	P No.		SEQ ID NO:	P No.		SEQ ID NO:	P No.
1	1		50	74		93	131
2	2		51	75		115	153
5	12		55	80		116	154
6	13		56	81		126	165
8	23		57	91		127	166
10	25		58	92		129	168
11	26		59	93		132	171
12	27		60	94		137	176

13	27B		61	95		138	177
14	27C		65	101		139	178
15	30		66	102		140	179
16	34		71	107		141	180
17	35		74	110		142	181
20	38		75	111		143	182
27	45		77	113		144	183
28	46		80	118		145	184
30	48		81	119		159	508
32	50		87	125		162	67
34	54		90	128		164	69
45	66		91	129			
46	70		92	130			

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention.